PROJECT NUMBER: 6906
PROJECT TITLE: Biological Effect of Smoke
PROJECT LEADER: J. M. Penn
WRITTEN BY: G. J. Patskan and J. M. Penn
PERIOD COVERED: April, 1988

I. PROTEIN KINASE C (PKC) WHOLE CELL ASSAY

A. **Objective**: To evaluate the effects of phorbol-12,13-dibutyrate (PDBu) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on 3T3 cells grown at various stages.

B. **Results**: Preliminary results indicate that there was a dose-related response with both PDBu and TPA. This dose-response effect was observed in both log phase cells and after the cells were refed with 0.5% serum media. However, there does not appear to be a single peak (i.e. Mw = 90,000) of increased phosphorylation. Additional software received this month may be helpful in quantitating the previously generated autoradiographs.

C. **Plans**: Complete the evaluation of autoradiographs using the new software. Repeat the dose-response experiment with PDBu and TPA while incorporating different procedures for gel and autoradiograph exposure, development and evaluation.

D. **Reference**:

Nixon, G. Notebook No. 8569, p. 112.

II. 3T3 CELL GROWTH CONDITIONS FOR THE PKC ASSAY

A. **Objective**: To define conditions required to generate "confluent/quiescent" cells.

B. **Results**: Two experiments were performed using cells that were grown in 10% serum and were then refed daily with media containing 5% serum. Although the cell numbers remained constant after the addition of 5% serum, a significant number of dead cells were observed. This suggests that there was concurrent cell growth and cell death which may not be indicative of the quiescent stage.

C. **Plans**: Evaluate other methods of inducing and confirming the quiescent stage for 3T3 cells.

D. **Reference**:

Nixon, G. Notebook No. 8569, pp. 112.

III. INHIBITION OF EGF BINDING ASSAY

A. **Objective**: To determine if reduced glutathione (GSH) has an effect on the activity of 2R1 IT CSC and catechol in the Inhibition of EGF Binding Assay.

B. **Results**: One experiment was conducted which examined the effects of GSH in the EGF assay. By themselves, 2R1 CSC and catechol inhibit EGF binding and reduce cell numbers. The addition of GSH (5mM) to CSC or catechol did not abrogate the effects of those agents. This preliminary data suggests that the constituents in CSC responsible for activity in the EGF assay do not react with GSH.

C. **Plans**: Repeat this experiment.

D. **Reference**:

Patskan, G. Notebook No. 8644, p. 30.

IV. PDBu BINDING ASSAY

A. **Objective**: Determine the effects of catechol on PDBu binding to 3T3 cells.

B. **Results**: In three experiments, a 19.5 hr exposure to catechol (0.01 - 10.0 µg/ml) caused a significant dose-dependent increase in the binding of ^{3}H-PDBu per cell. In two experiments, catechol elicited no significant effects when added to the cells at the time of binding.

C. **Plans**: Examine the effects of compounds that inhibit protein kinase C (PDBu receptor) activity.

D. **Reference**:

Ferguson, T. J. Notebook No. 8645, p. 45.